

Rat Nedd4

Figure1

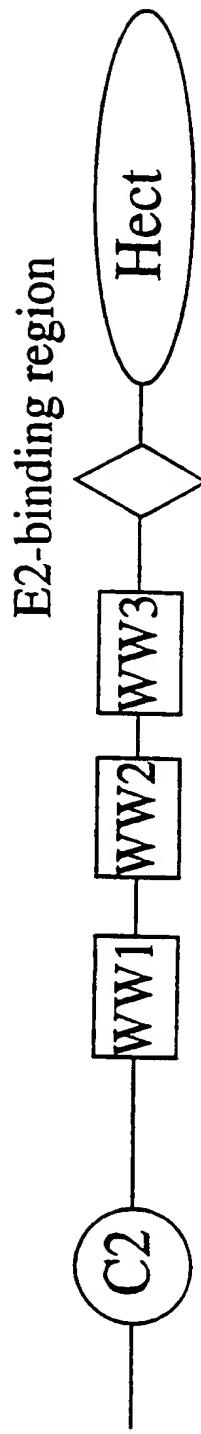
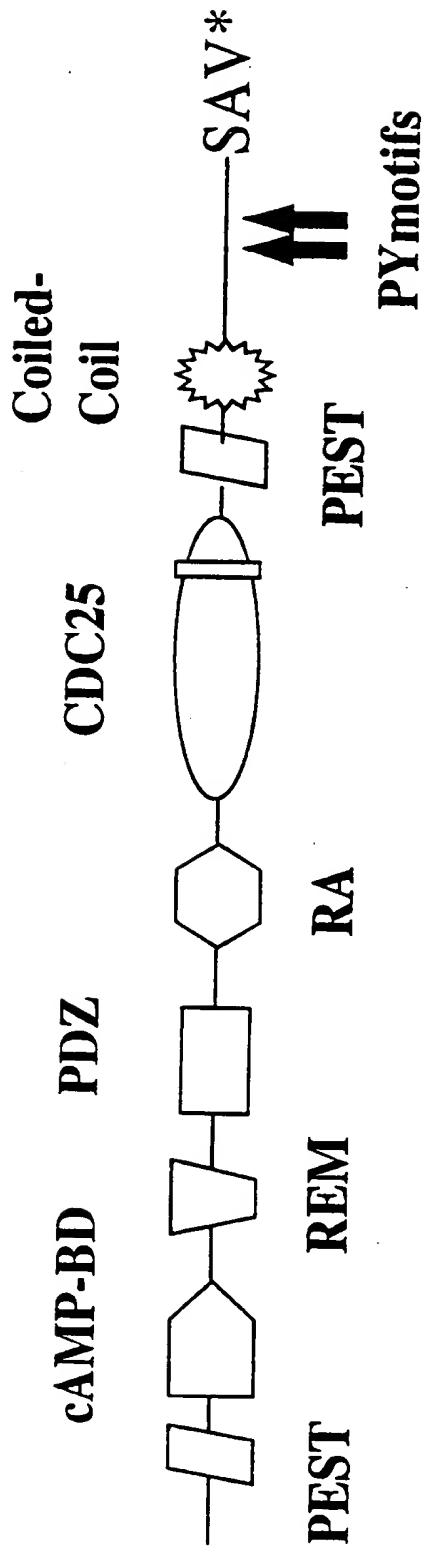


Figure 2: Clone 7.7 is the homolog of human clone KIAA0313

- Clone 7.7 exhibits 75% identity and 95% similarity of human clone KIAA0313.

Figure 3A Schematic Diagram of GRF4



4618 b.p.

1/1 31/11
atg aaa cca cta gca atc cca gct aac cat gga gtt atg ggc cag cag gag aaa cac tca
M K P L A I P A N H G V M G Q Q E K H S
61/21 91/31
ctt cct gca gat ttc aca aaa ctg cat ctt act gac agt ctc cac cca cag gtg acc cac
L P A D F T K L H L T D S L H P Q V T H
121/41 151/51
gtt tct tct agc cat tca gga tgt atg act agt gat tct ggg agc agc agt ctt tct
V S S S H S G C S I T S D S G S S S L S
181/61 211/71
gat atc tac cag gcc aca gaa agc gag gct ggt gat atg gac ctg agt ggg ttg cca gaa
D I Y Q A T E S E A G D M D L S G L P E
241/81 271/91
aca gca gtg gat tcc gaa gac gac gat gaa gaa gac att gag aga gca tca gat cct
T A V D S E D D D D E E D I E R A S D P
301/101 331/111
ctg atg agc agg gac att gtg aga gac tgc cta gag aag gac cca att gac cgg aca gat
L M S R D I V R D C L E K D P I D R T D
361/121 391/131
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D D I E Q L L E F M H Q L P A F A N M T
421/141 451/151
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M S V R R E L C A V M V F A V V E R A G
481/161 511/171
acc ata gtg tta aat gat ggt gaa gag ctc tgg tca gtg att ctc aat gga tct
T I V L N D G E E L D S W S V I L N G S
541/181 571/191
gtt gaa gtg act tat cca gat gga aaa gca gaa ata ctg tgc atg gga aat agt ttt ggt
V E V T Y P D G K A E I L C M G N S F G
601/201 631/211
gtc tct cct acc atg gac aaa gaa tac atg aaa gga gtg atg aga aca aag gtg gat gac
V S P T M D K E Y M K G V M R T K V D D
661/221 691/231
tgc cag ttt gtc tgc ata gcc cag caa gat tac tgc cgt att ctc aat caa gta gaa aag
C Q F V C I A Q Q D Y C R I L N Q V E K
721/241 751/251
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N M Q K V E E E G E I V M V K E H R E L
781/261 811/271
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D R T G T R K G H I V I K G T S E R L T
841/281 871/291
atg cat ttg gtg gaa gag cat tca gta gta gat cca aca ttc ata gaa gac ttt ctg ttg
M H L V E E H S V V D P T F I E D F L L
901/301 931/311
acc tat agg act ttt ctt tct agc cca atg gaa gtg ggc aaa aag tta ttg gag tgg ttt
T Y R T F L S S P M E V G K K L L E W F
961/321 991/331
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N D P S L R D K V T R V V L L W V N N H
1021/341 1051/351
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F N D F E G D P A M T R F L E E F E N N

cNMP-BD

REM

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 A K R R L M T L T K P S R E A P L P F I
 1201/401 1231/411
 tta ctt gga ggc tct gag aag gga ttt gga atc ttt gtt gac agt gta gat tca ggt agc
 L L G G S E K G F G I F V D S V D S G S
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 F E N I O L S K A M E I L R N N T H L S
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 atc act gtg aaa acc aat tta ttt gta ttt aaa gaa ctt cta aca aga ttg tca gaa gag
 I T V K T N L F V F K E L L T R L S E E
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 K R N G A P H L P K I G D I K K A S R Y
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 S I P D L A V D V E Q V I G L E K V N K
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 K S K A N T V G G R N K L K K I L D K T
 1621/541 1651/551
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 R I S I L P Q K P Y N D I G I G Q S Q D
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 D S I V G L R Q T K H I P T A L P V S G
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 T L S S S N P D L L Q S H H R I L D F S
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 A T P D L P D Q V L R V F K A D O O S R
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 Y I M I S K D T T A K E V V I Q A I R E
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 F A V T A T P D O Y S L C E V S V T P E
 1981/661 2011/671
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 G V I K Q R R L P D Q L S K L A D R I Q
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 ctg agt gga agg tat tat ctg aaa aac aac atg gaa aca gaa act ctt tgt tca gat gaa
 L S G R Y Y L K N N M E T E T L C S D E
 2101/701 2131/711
 gat gct cag gag ttg ttg aga gag agt caa att tcc ctc ctt cag ctc agc act gtg gaa
 D A Q E L L R E S Q I S L L O L S T V E
 2161/721 2191/731
 gtt gca aca cag ctc tct atg cga aat ttt gaa ctc ttt cgc aac att gaa cct act gaa
 V A T Q L S M R N F E L F R N I E P T E

PDZ

RA

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Y I D D L F K L R S K T S C A N L K R F
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E E V I N Q E T F W V A S E I L R E T N
 2341/781 2371/791
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Q L K R M K I I K H F I K I A L H C R E
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C K N F N S M F A I I S G L N L A P V A
 2461/821 2491/831
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 2581/861 2611/871
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N L Q P P I I P L F P V I K K D L T F L
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 cac gaa gga aat gac tca aaa gta gac ggg ctg gtc aat ttt gag aag cta agg atg att
H E G N D S K V D G L V N F E K L R M I
 2701/901 2731/911
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A K E I R H V G R M A S V N M D P A L M
 2761/921 2791/931
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F R T R K K K W R S L G S L S Q G S T N
 2821/941 2851/951
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A T V L D V A Q T G G H K K R V R R S S
 2881/961 2911/971
 ttt ctc aat gcc aaa aag ctt tat gaa gat gcc caa atg gct cga aaa gtg aag cag tac
F L N A K K L Y E D A Q M A R K V K Q Y
 2941/981 2971/991
 ctt tcc aat ttg gag cta gaa atg gac gag gag agt ctt cag aca tta tct ctg cag tgt
L S N L E L E M D E E S L O T L S L O C Continuation of the
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E P A T N T L P K N P G D K K P V K S E
 3061/1021 3091/1031
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T S P V A P R A G S Q Q K A Q S L P Q P
 3121/1041 3151/1051
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 3181/1061 3211/1071
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L Y P S R K K V P V K D L P P F G I N S
 3241/1081 3271/1091
 cca caa gct tta aaa aaa att ctt tct ttg tct gaa gaa gga agt ttg gaa cgt cac aag
P Q A L K K I L S L S E E G S L E R H K
 3301/1101 3331/1111
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K Q A E D T I S N A S S Q L S S P P T S

Insertion
Unique to
GRF4

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3361/1121 3391/1131
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 P Q S S P R K G Y T L A P S G T V D N F
 3421/1141 3451/1151
 tca gat tct ggt cac agt gaa att tct tca cga tcc agt att gtt agc aat tcg tct ttt
 S D S G H S E I S S R S S I V S N S S F
 3481/1161 3511/1171
 gac tca gtg cca gtc tca ctg cac gat gag agg cgc cag agg cat tct gtc agc atc gtg
 D S V P V S L H D E R R Q R H S V S I V
 3541/1181 3571/1191
 gaa aca aac cta ggg atg ggc agg atg gag agg cgg acc atg att gaa cct gat cag tat
 E T N L G M G R M E R R T M I E P D Q Y
 3601/1201 3631/1211
 agc ttg ggg tcc tat gca cca atg tcc gag ggc cga ggc tta tat gct aca gct aca gta
 S L G S Y A P M S E G R G L Y A T A T V
 3661/1221 3691/1231
 att tct tct cca agc aca gag gaa ctt tcc cag gat cag ggg gat cgc gcg tca ctt gat
 I S S P S T E E L S Q D Q G D R A S L D
 3721/1241 3751/1251
 gct gct gac agt ggc cgt ggg agc tgg acg tca tgc tca agt ggc tcc cat gat aat ata
 A A D S G R G S W T S C S S G S H D N I
 3781/1261 3811/1271
 cag acg atc cag cac cag aga agc tgg gag act ctt cca ttc ggg cat act cac ttt gat
 Q T I Q H Q R S W E T L P F G H T H F D
 3841/1281 3871/1291
 tat tca ggg gat cct gca ggt tta tgg gca tca agc agc cat atg gac caa att atg ttt
 Y S G D P A G L W A S S S H M D Q I M F
 3901/1301 3931/1311
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 4021/1341 4051/1351
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 4081/1361 4111/1371
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 L T S V T T E E T K P V P M P A H I A V
 4141/1381 4171/1391
 gca tca agt act aca aag ggg ctc att gca cga aag gag ggc agg tat cga gag ccc ccc
 A S S T T K G L I A R K E G R Y R E P P
 4201/1401 4231/1411
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 P T P P G Y I G I P I T D F P E G H S H
 4261/1421 4291/1431
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 P A R K P P D Y N V A L Q R S R M V A R
 4321/1441 4351/1451
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 S S D T A G P S S V Q Q P H G H P T S S
 4381/1461 4411/1471
 agg cct gtg aac aaa cct cag tgg cat aaa ccg aac gag tct gac ccg cgc ctc gcc cct
 R P V N K P Q W H K P N E S D P R L A P

PY motifs

(continued next page)

4441/1481

4471/1491

tat cag tcc caa ggg ttt tcc acc gag gag gat gaa gat gaa caa gtt tct gct gtt tga
Y Q S Q G F S T E E D E D E Q V S A V *

4501/1501

4531/1511

ggc aca gac ttt tct gga agc aga gcg agc cac ctg aaa gga gag cac aag aag acg tcc
G T D F S G S R A S H L K G E H K K T S

4561/1521

4591/1531

tga gca ttg gag cct tgg aac tca cat tct gag gac ggt gga cca gtt tgc ctc ctt c

* A L E P W N S H S E D D G G P V C L L

PDZ binding motif

00354736 0022000

CDC25 Domain

Figure 4

Figure 5: GRF4-REM domain

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Figure 6A:

**Overall structure comparison between
GRF4 and other known mammalian
RasGEF/RasGRF**

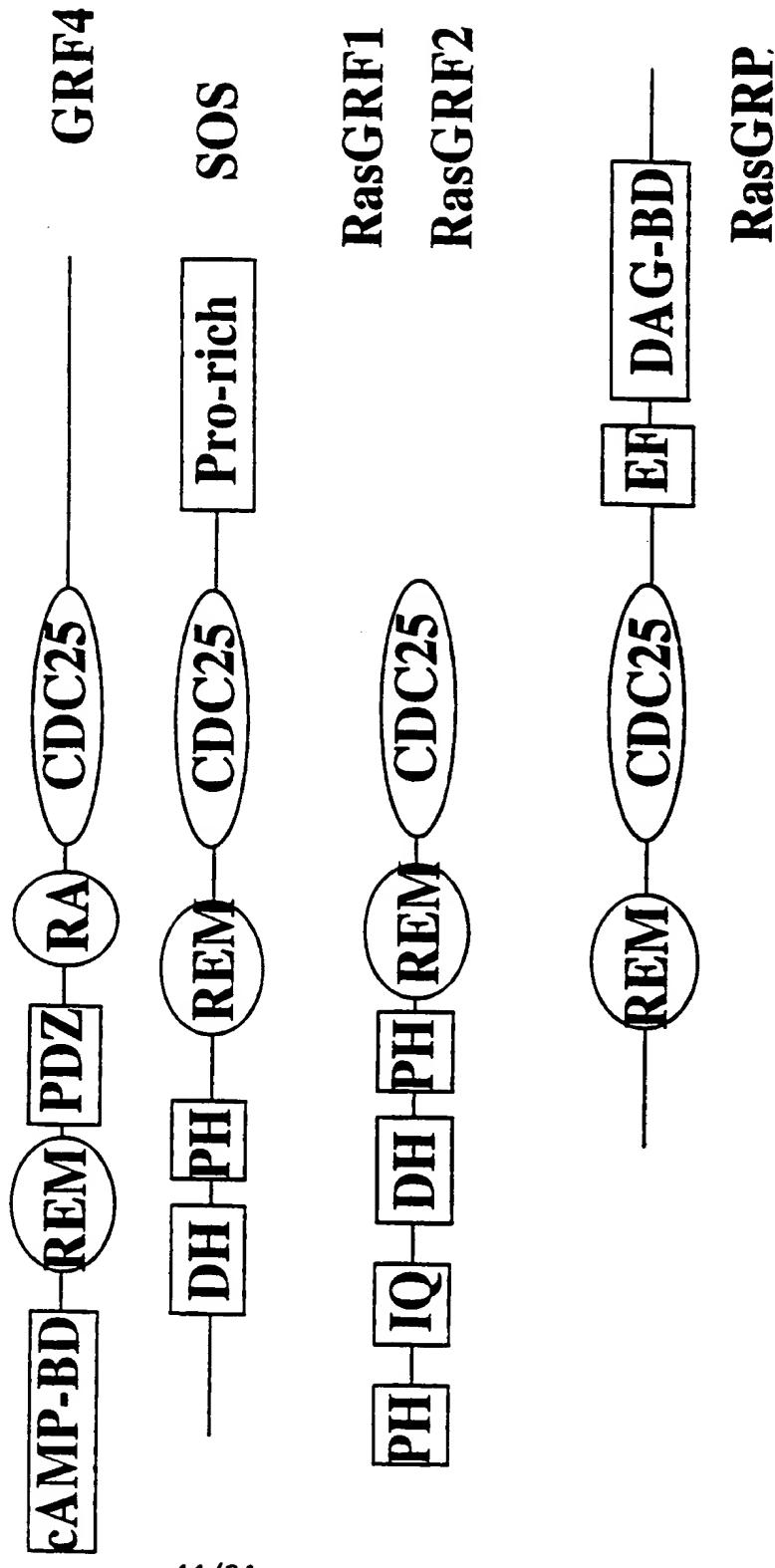


Fig.6B

Ras Signaling pathway

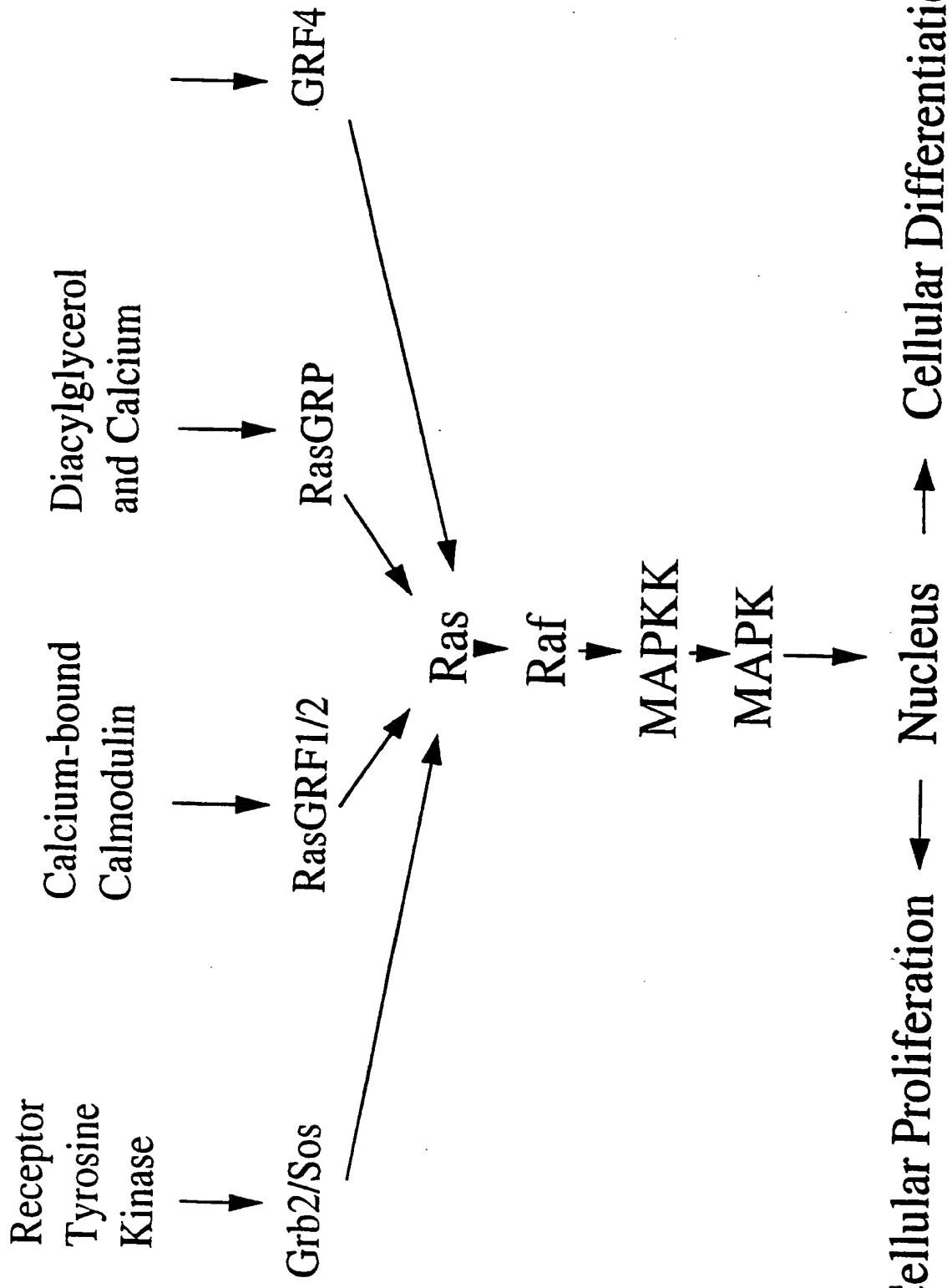


Figure 7

PDZ domain

13/34

hGFR4	LTKPSREAPLPPFILLGGSEK-----	-GFGIFVVDSSGSKATEAG-LKRGDQIL
dGFR4	LTRSSRDEPLNFRIVGGYELRGVAIATGNAAVGIVYISHVEPGSKAQDVG-LKRGDQIH	
hPTP-BAS-1	NLKKDAKYGLGFQIIGGEKMGRLL-----	-DLGIFISSVAPGGPADLDGCLKGDRLL
hPSD-95	IVIHRGSTGGLFNVGGEDG-----	-EGIFISFILAGGPADLSGEELRKGDQIL
rLin-7-C	VELPKTEEGLGFNIMGGKEQ-----	-NSPIIYISRIIPGGIADRHHGGLKRGDQLL
hDGL	VKVQKGSEPLGISIVSGEKG-----	-GIYVSKVTVGSIAHQAG-LEYGDQLL
	.* : : : . * .	* : : . * . * . * . * . * : :
hGFR4	EVNGQNFENIQLSKAMEILR	
dGFR4	EVNGQSLDHVTSKRALEILT	
hPTP-BAS-1	SVNSVSLEGVSHHAAIEILQ	
hPSD-95	SVNGVDLNRNASHEQAAIALK	
rLin-7-C	SVNGVSVEGEHHIEKAVELLK	
hDGL	EFNGINLRSATEQQARLIG	
	..* . . .	* : :

Figure 8

cNMP-BD

hGRF4	MVFAVVVERAGTIVLNDGEELDSWSVILNGSVEV	---	TYPDGKAELCMGNNSFGVSPTMDK
dGRF4	MVFAVVVDKAGTVVMSDGEELDSWSVILINGAVEI	---	EHANGSREELQMGDSFGILPTMDK
hEPAC	LIFEPEPHSKAGTVLFSQGDKGTSWYIIWKGSVNVV	---	THGKGLVTTLHEGDDFGQLALVND
PRKAR1B	AMFPVTHIAGETVIQQGNNEGDNFYVVDQGEVDVY	---	VNGEWVTN-ISEGGSFGELALIYG
hPKGII	CMYGRNYQQGSYIIKQGEPGNHTIFVLAEGRLEV	---	FQGEKLLSSIPMWTTFGEELAIIYN
m-EAG	RLRSVVYLPNDYVCKKGEIGREMYIIQAGQVQVQLGGPDGKSVLVTLKAGSVFGEISLLAV	:	.*: : * : : * : * : * : :
hGRF4	---EYMKGVMRKTVDDCQFVCIAQQDYCRILNQVEKNMQKVEEE		
dGRF4	---LYHHRGVMRKCDQFVCITQTDYRILQHQGEENTRRHEDE		
hEPAC	---APRAATIILREDDNCHFLRVDKQDFNRIIKDVEAKTMREEH		
PRKAR1B	---TPRAATVKAKTD-LKLWGDIDRSYRRILMGSTLRKRMYEE		
hPKGII	---CTRTASVKAITN-VKTTWALDREVFQNIMRRTAQARDEQYRN		
m-EAG	GGGNRRRTANVVAHGF-TNLFILDKKDLNEILVHYPESQKLLRK		.*: : : : * .*

Figure 9: GRF4-RA domain

dgk-1a_ce_	-----REDFE---IIRVF DGNNS---YRSQIS-----RNTIVAKHVSQQV RDAALR
GRF4	-HHRILDFS---ATPDLPDQVL RVFKA DQQS-----RYIMISKDTAKEVVTQAIR
Ra1GDS_h_	SILVTSQDKAPSVISRVLKKNNRDSAVASEYELVQOLLPGERELTIPASANVFYAMDGASH
	*
dgk-1a_ce_	RFHI--NDT PERYYIT-QVVGEV EEEILED----PVP-----LRNVKRP EKGRAQIFIR
GRF4	EFAV--TATPDQYSLC-EVSVTPEGV I KQR---RLP-----DQLSK--LADRIQLSGR
Ra1GDS_h_	DFLIRHGE GPLLH ASPVARL P QELL R VREEGAPFP GSRPQGGRLHGH CSEE EAPL AYR
	*
dgk-1a_ce_	YYD-----
GRF4	YYLKNNME-
Ra1GDS_h_	SHGVHTRCG

15/34

Figure 10

Tissue Distribution of GRF4

A: Rat multiple tissues mRNA blot

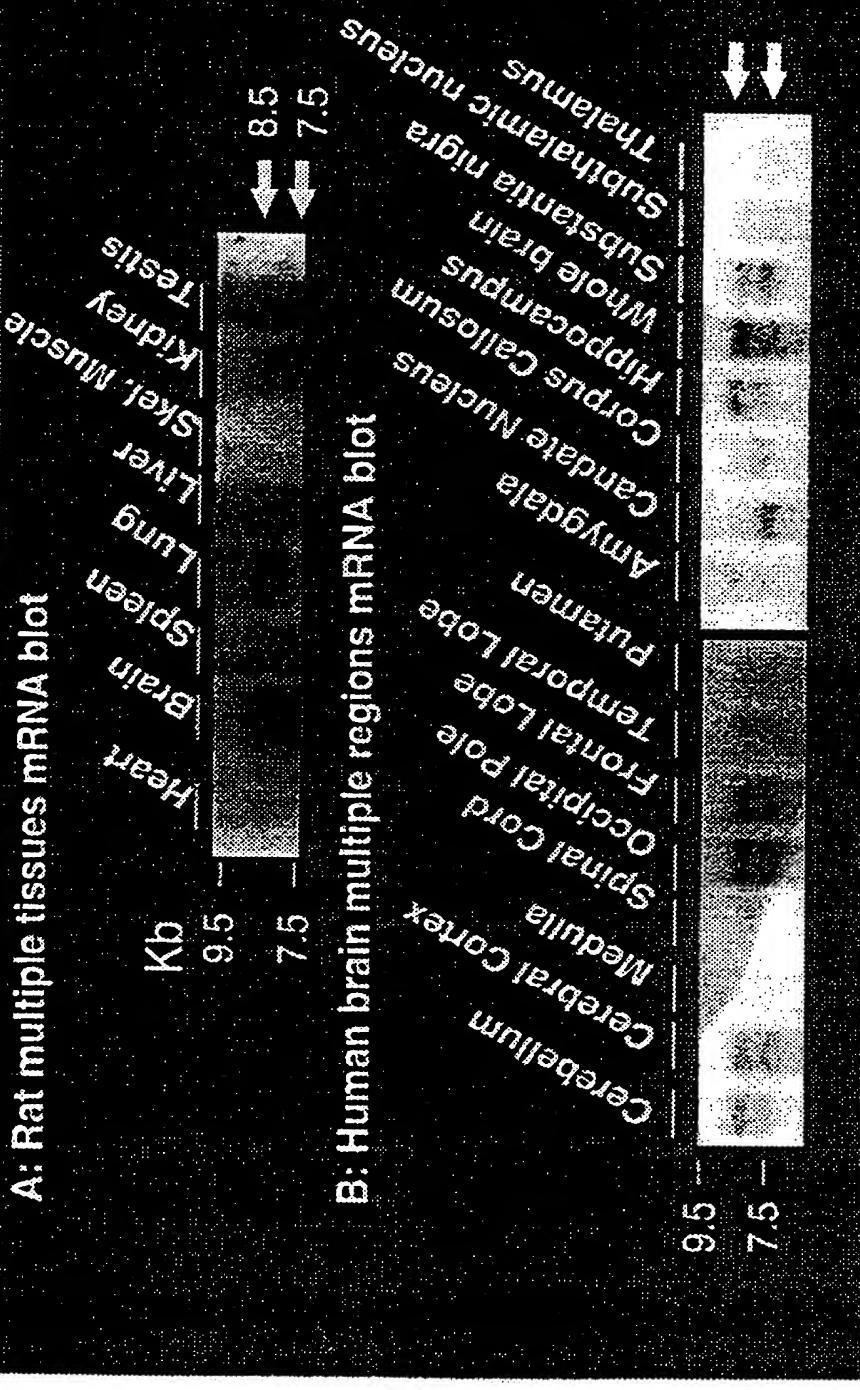


Figure 11

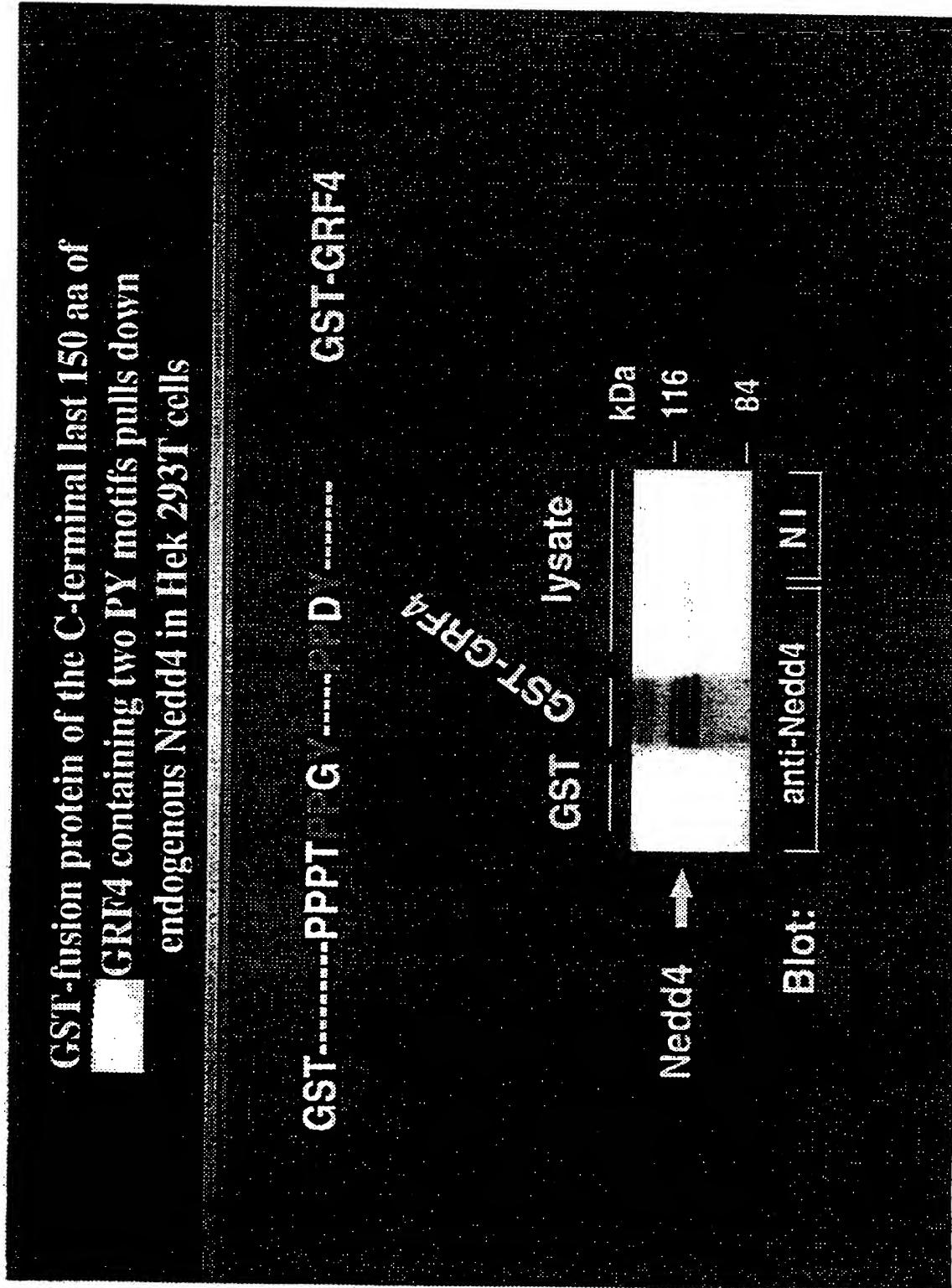


Figure 12

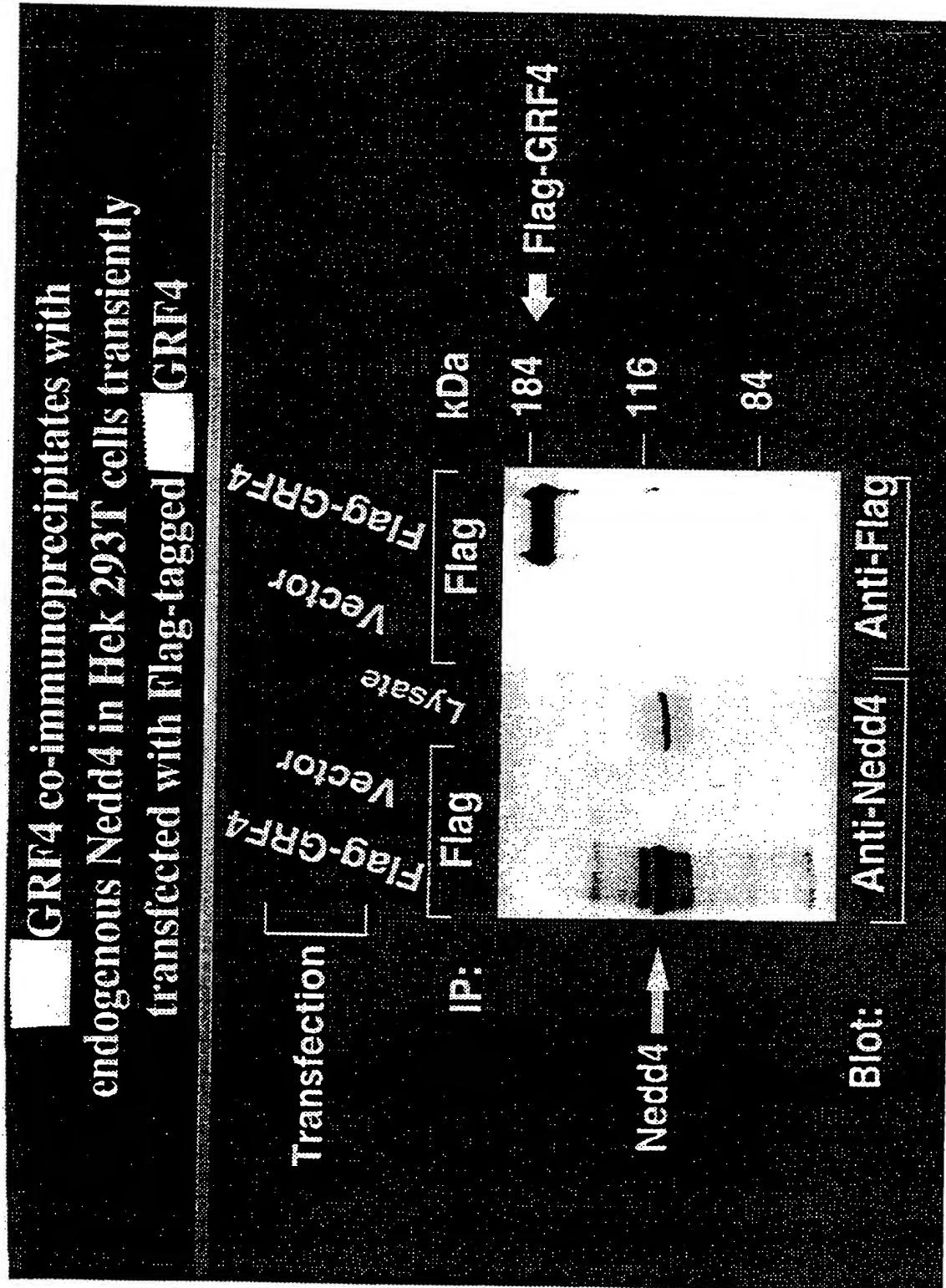


Fig.13

In-vitro GEF assay

WO 00/43510

PCT/CA00/00042

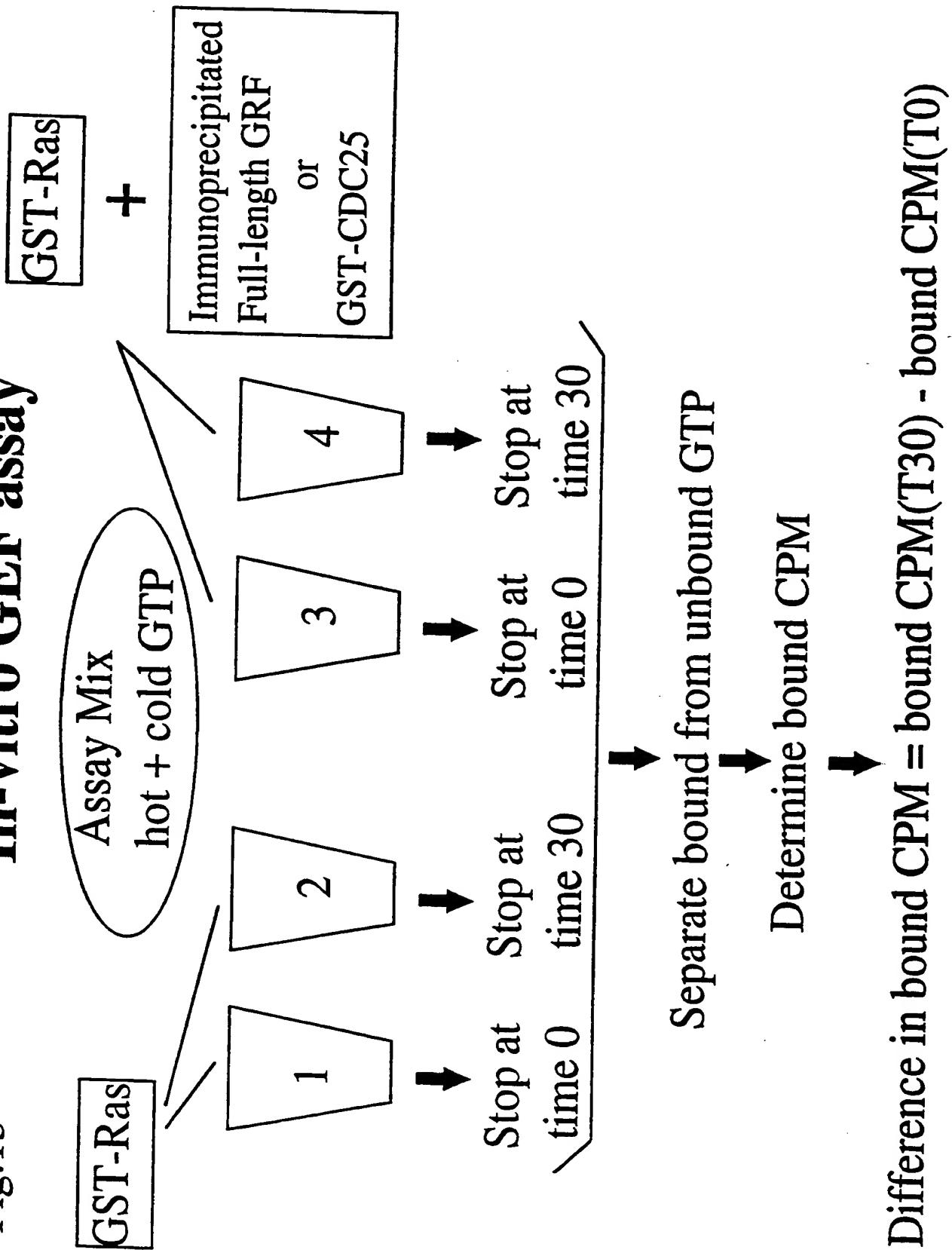


Fig.14 GRF4 is active on Ras : in-vitro GEF assay using immunoprecipitated full-length GRF4

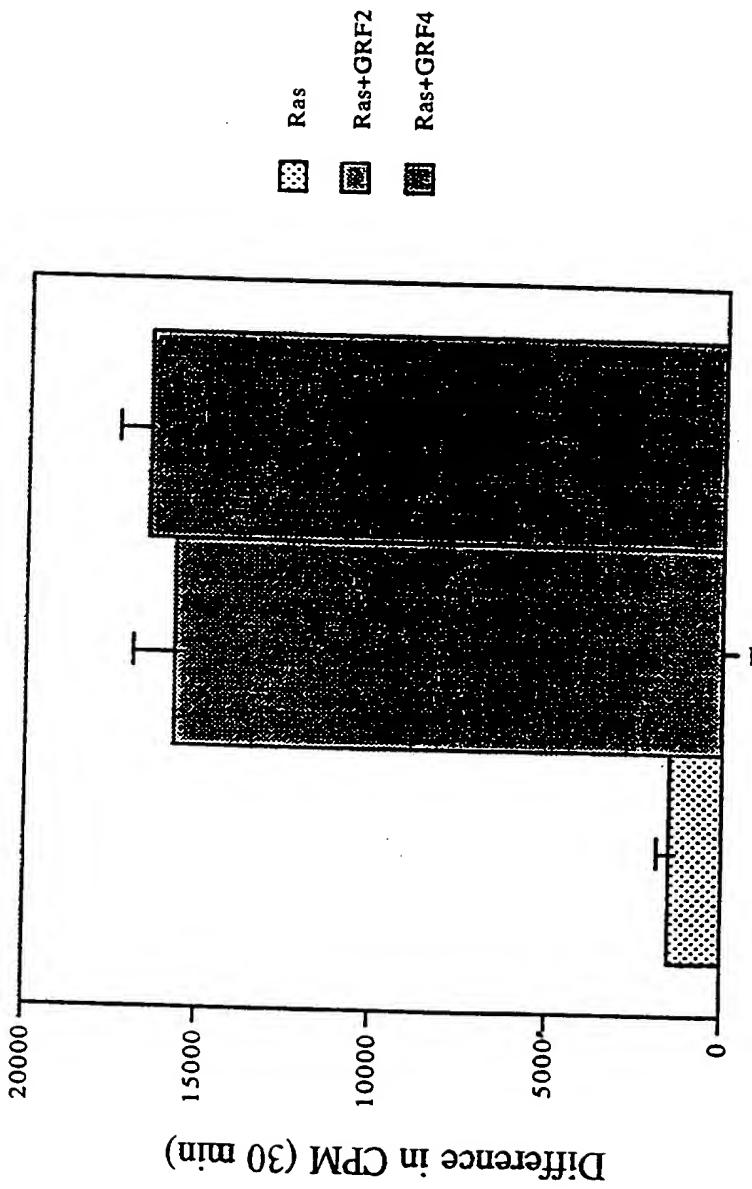


Figure 15

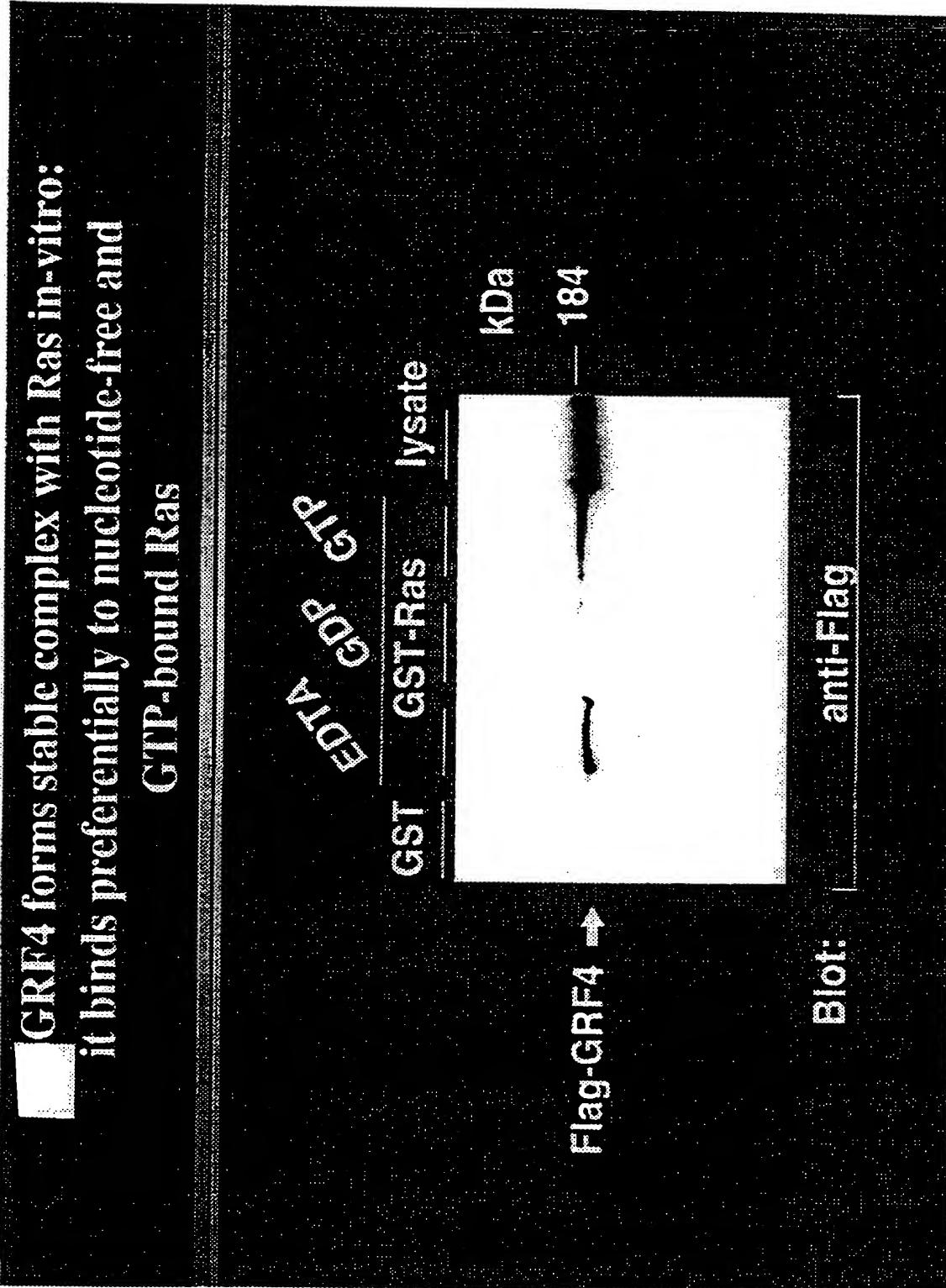


Figure 16

■ Transformation Assay: ■ GFR4 induces foci formation in Rat2 fibroblasts

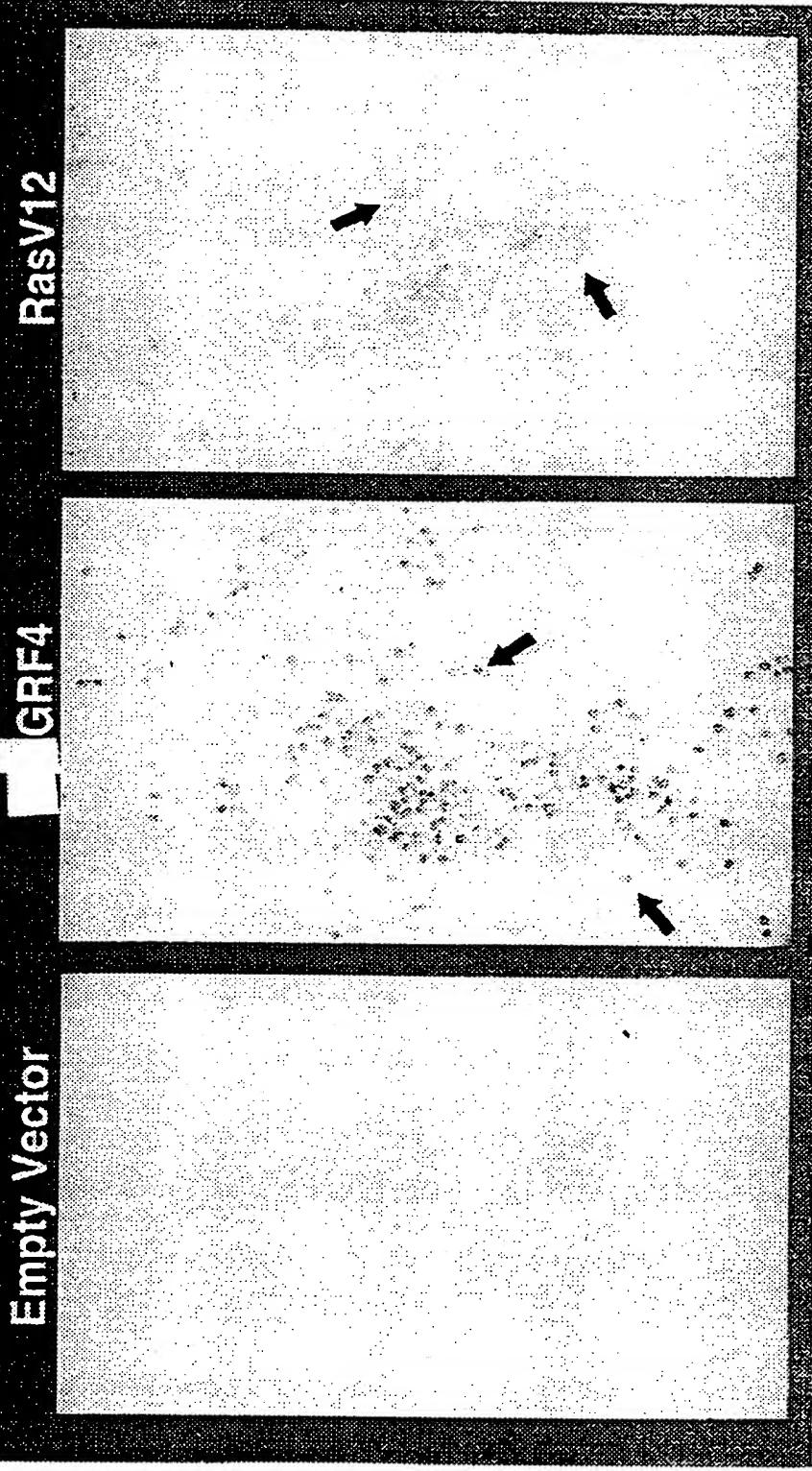


Figure 17

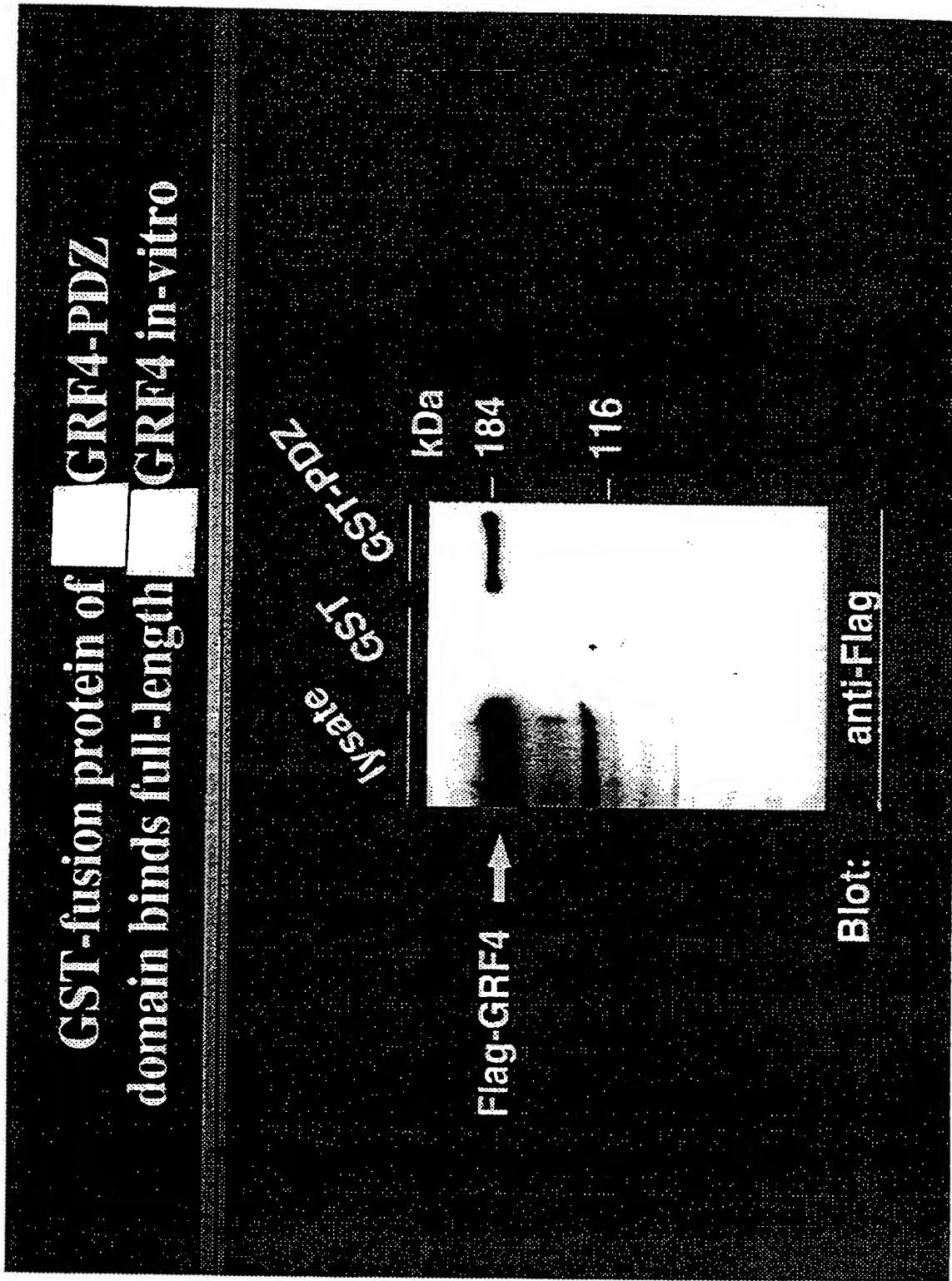
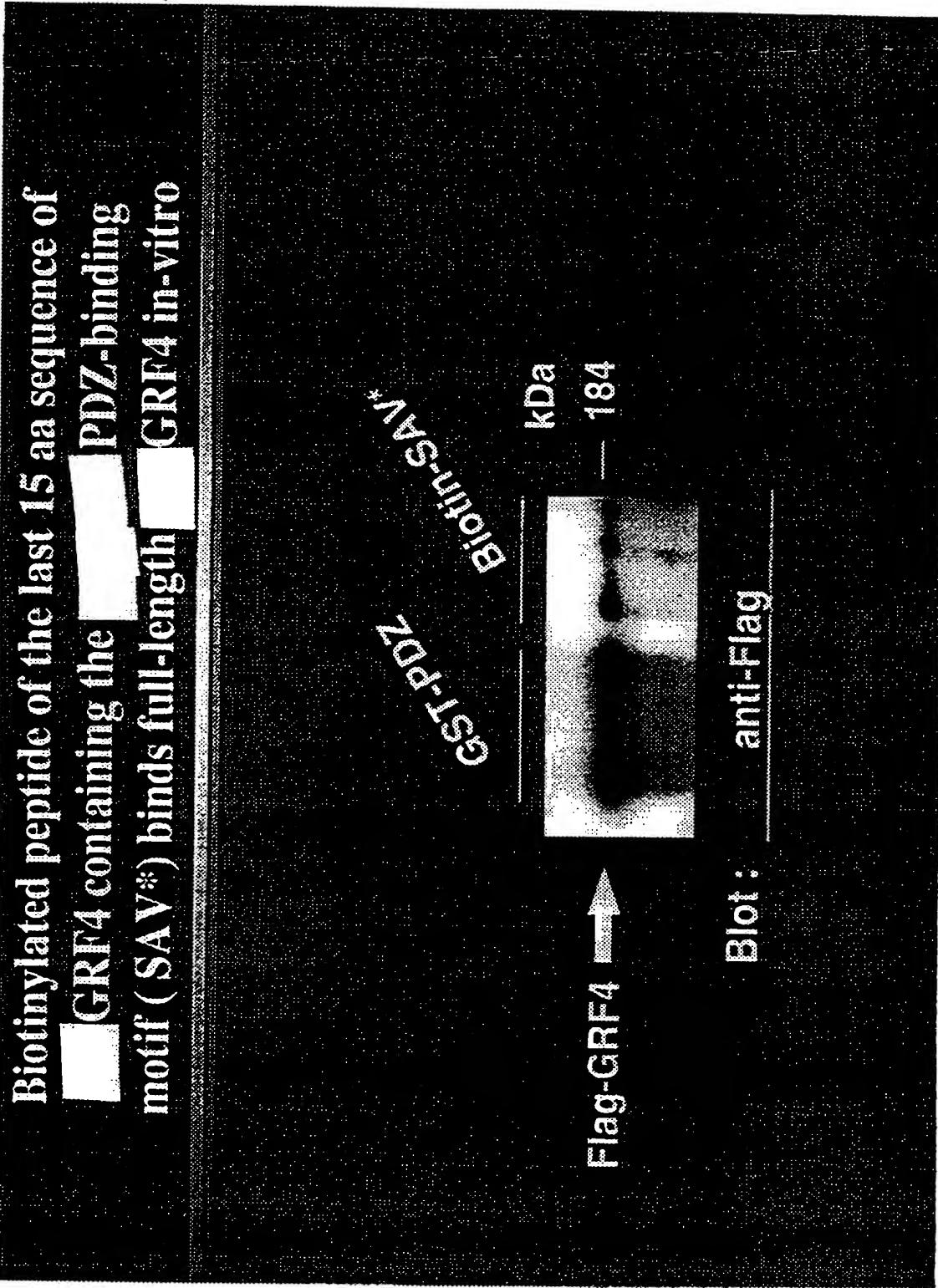


Figure 18



6568 bp
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DDCQFVCAQQDYCRILNQVEKNMQKVEEEGEIVMVKEHRELDRTGTRKGHIVIKGTS
ERLTMHVEEHSSVVDPTFIEDFLTYRTFLSSPMEVGKKLEWFNDPSLRDKVTRVVL
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REAPLPIIILGGSEKGFGIFVDSVDSGSKATEAGLKRGDQILEVNGQNFENIQLSKAM
EILRNNNTHLSITVTKTNLFVFKEELLTRSEEKRNGAPHLPKIGDIKKASRYSIPDLAVD
VEQVIGLEVKVNKKSKANTVGGGRNKLKKILDKTRISILPQPKYNDIGQSQDDSVGL
RQTKHIPTALPVSGTLLSSNPDLQLQSHRILDFTAATPDLPDQVLRVFKADQQSRYIMI
SKDTTAKEVVIQAIREFAVTATPDQYSLCEVSVTPEGVIKQRRLPDQLSKLADRIQLS
GRYYLKNNMETETLCSDEDAQELLRESQISLLQLSTVEVATQLSMRNFELFRNIEPTE
YIDDLFKLRSKTSCANLKRFEEVINQETFWVASEILRETNQLKRMKIIKHFIFKIALHC
RECKNFNFSMFAIISGLNLPAPLARLRTTWEKLPNPKYEKLQFDLQDFPSRNMAKYRNV
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63. .4562

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ORIGIN

(continued next page)

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 1981 agttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt
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 2281 aatgtttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt
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 2461 aatgtttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt
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4681 cctgccttaa aagcagcatg gggcttcttc tcccccctt cttttccctt tgcatgtga
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4981 agaaaagctt gccatgttac tttttttttt tttttttttt ccaaggaga caaagaaaaaa
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801 b.p.

1/1 31/11
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 T K G N K S W S S T A V A A A L E L V D
 L K G T K A G A P P R W R P L * N * W I
 * R E Q K L E L H R G G G R S R T S G S

61/21 91/31
 CCC CCG GGC TGC AGG AAT TCA AGC GGT GGG AAG GAT GTC TCC GCT GAG GCA GAG AGC AGC
 P P G C R N S S G G K D V S A E A E S S
 P R A A G I Q A V G R M S P L R Q R A A
 P G L Q E F K R W E G C L R * G R E Q Q

121/41 151/51
 AGC ATG GTG CCC GTG ACT ACA GAG GAA GCC AAA CCT GTC CCT ATG CCT GCC CAC ATA GCT
 S M V P V T T E E A K P V P M P A H I A
 A W C P * L Q R K P N L S L C L P T * L
 H G A R D Y R G S Q T C P Y A C P H S C

181/61 211/71
 GTG ACG CCG AGC ACT ACC AAG GGA CTC ATC GCA CGG AAG GAA GGC AGG TAC CGG GAG CCG
 V T P S T T K G L I A R K E G R Y R E P
 * R R A L P R D S S H G R K A G T G S R
 D A E H Y Q G T H R T E G R Q V P G A A

241/81 271/91
 CCT CCC ACA CCT CCA GGC TAC GTG GGC ATC CCC ATT GCC GAT TTC CCA GAA GGG CCT TGC
 P P T P P G Y V G I P I A D F P E G P C
 L P H L Q A T W A S P L P I S Q K G L A
 S H T S R L R G H P H C R F P R R A L P

301/101 331/111
 CAC CCG GCC AGG AAG CCC CCG GAT TAC AAC GTG GCC CTG CAG CGG TCC CGC ATG GTG GCA
 H P A R K P P D Y N V A L Q R S R M V A
 T R P G S P R I T T W P C S G P A W W H
 P G Q E A P G L Q R G P A A V P H G G T

361/121 391/131
 CGG CCC ACT GAG GCC CCG GCA CCG GGC CAG ACG CGG CCT GCA GCC GCA GCC AGC CGG CCG
 R P T E A P A P G Q T P P A A A A S R P
 G P L R P R H R A R R R L Q P Q P A G R
 A H * G P G T G P D A A C S R S Q P A G

421/141 451/151
 GGC AGC AAG CCA CAG TGG CAC AAG CCC AGC GAC GCA GAC CCA CGC CTC GCG CCC TTC CAG
 G S K P Q W H K P S D A D P R L A P F Q
 A A S H S G T S P A T Q T H A S R P S S
 Q Q A T V A Q A Q R R R P T P R A L P A

481/161 511/171
 CCG CAG GCT TCG CAG GAG CGG AGG ACG AAG ATG AAC AAG TGT CTG CTG TTT GAG GCG
 P Q A S Q E R R R T K M N K C L L F E A
 R R L R R S G G G R R * T S V C C L R R
 A G F A G A E E D E D E Q V S A V * G A

541/181 571/191
 CAG GCT CCT TGA TCC ACA GTG AGC CAC CCA AAG GAG AGC ACA AGA AGA CGT CCC AAG CCT
 Q A P * S T V S H P K E S T R R R P K P
 R L L D P Q * A T Q R R A Q E D V P S L

601/201 631/211
 TGG AGC CTT GGC ACG CAC ATC TGA GGA TGG TGG ACC AGT TTG CCT CCT TCC CTG CCT TAA
 W S L G T H I * G W W T S L P P S L P *
 G A L A R T S E D G G P V C L L P C L K
 E P W H A H L R M V D Q F A S F P A L K

661/221 691/231
 AGC AGC ATG GGG CTT CTT CTC CCC TTC TTC CTT TCC CCT TTG CAT GTG AAA TAC TGT GAA
 S S M G L L L P F F L S P L H V K Y C E
 A A W G F F S P S S F P L C M * N T V K
 Q H G A S S P L L P F P F A C E I L * R

(continued next page)

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Figure 19B

721/241

GAA ATT GCC CTG GCA CTT TGC AGA CTT GTT GCT TGA AAT GCA CAG CCC AGC AGC CCC TGA
S I A L A L C R L V A * N A Q P S S P .
K L P W H F A D L L L E M H S P A A P E
N C P G T L Q T C C L K C T A Q Q P L S
781/261
GCT GCT GCC TGC CAC GTC ACG [SEQ ID NO: 3]
A A A C H V T [SEQ ID NO: 4]
L L P A T S [SEQ ID NO: 5]
C C L P R H [SEQ ID NO: 6]

The PDZ domain (but not the C terminal SxV motif)
is required for plasma membrane localization of
GRF4

GRF4 Localization

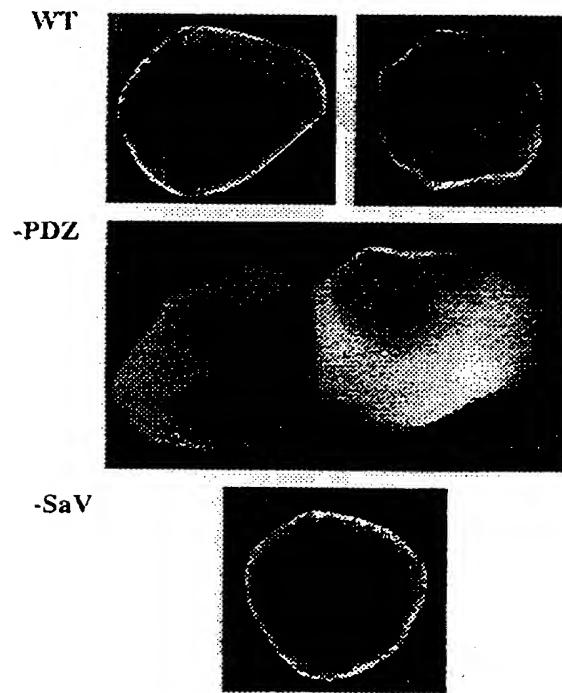


Figure 20

Figure 21

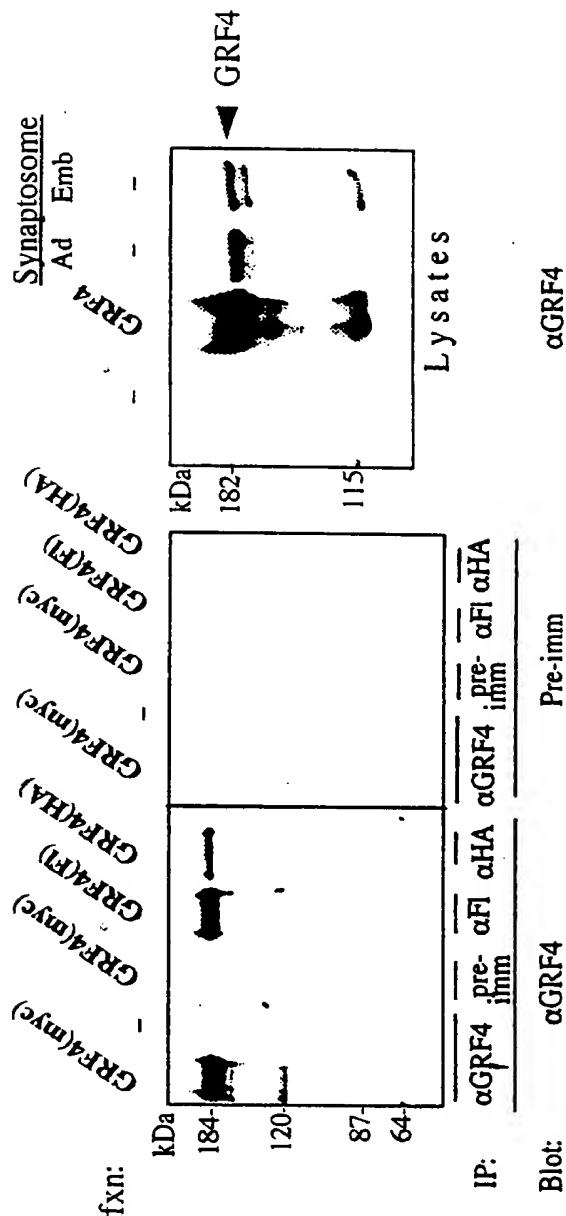
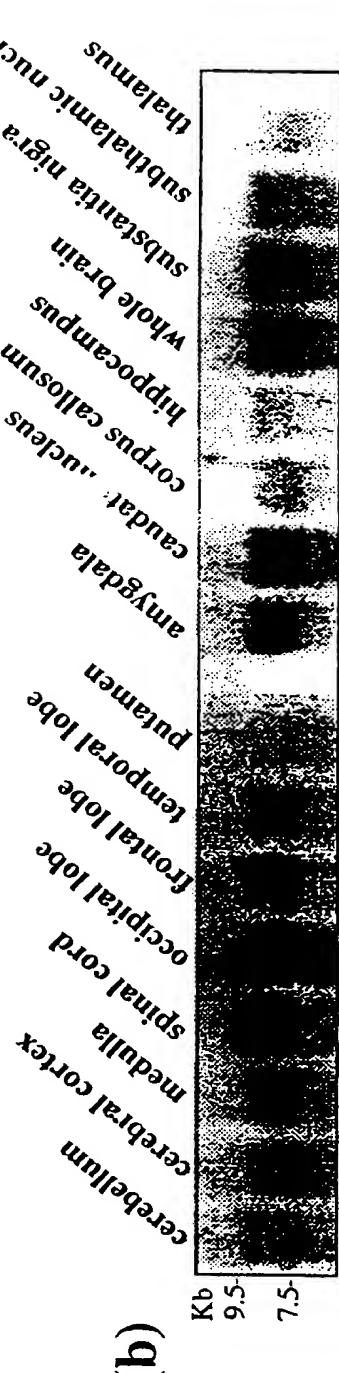
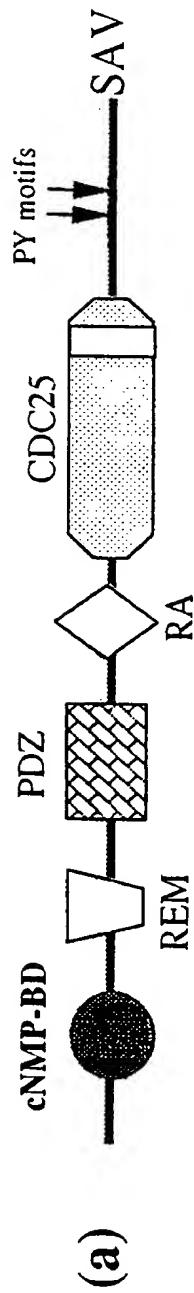


Figure 22

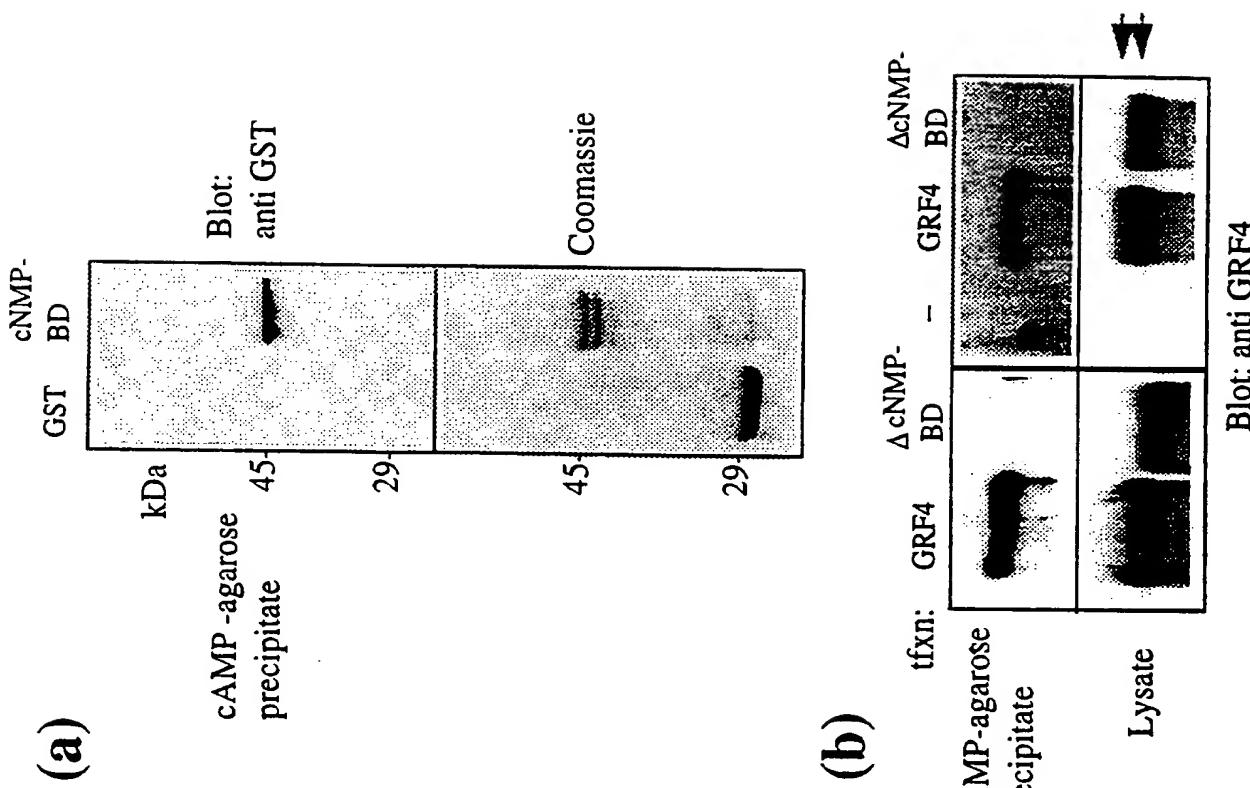
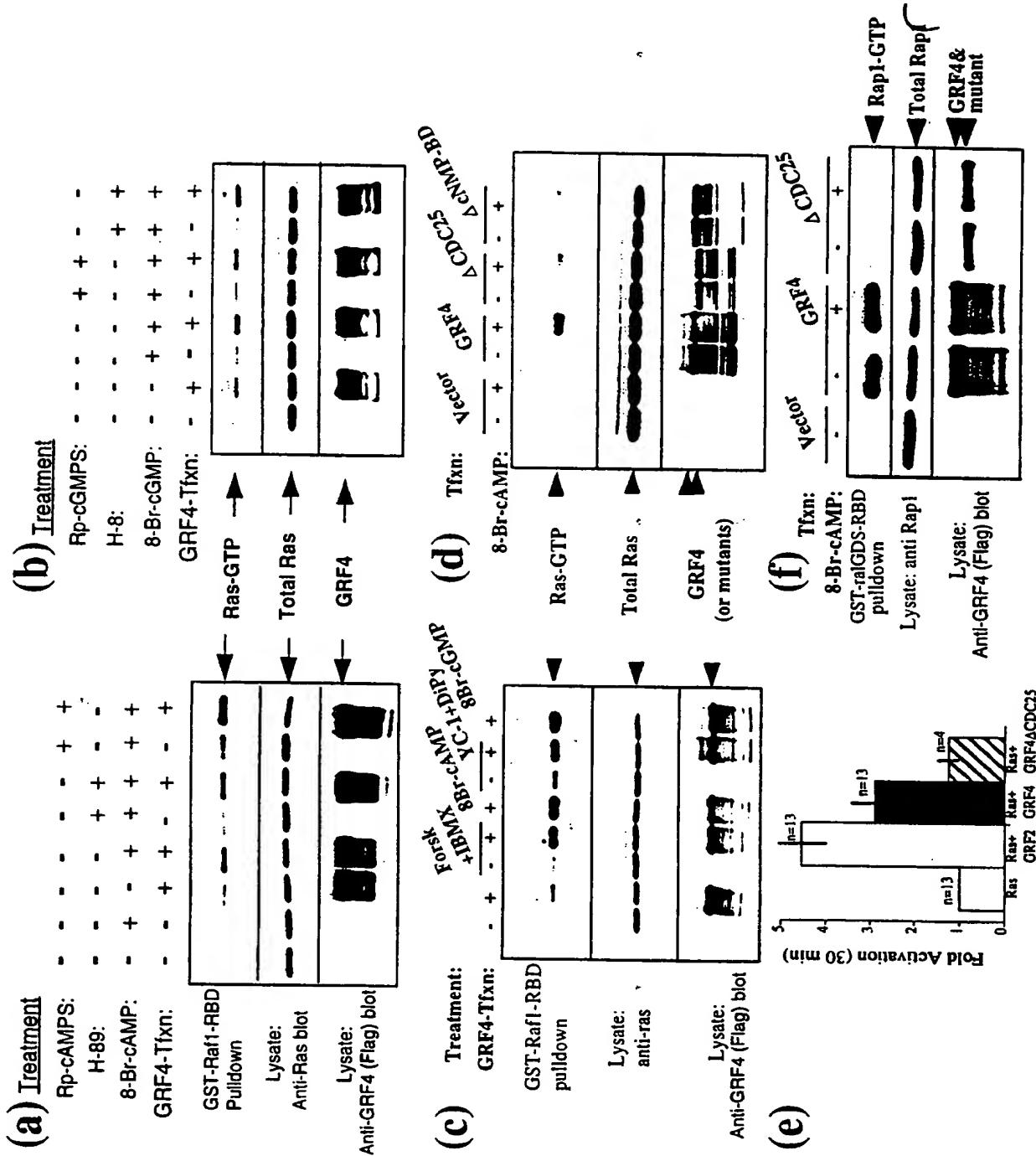


Figure 23



33/34

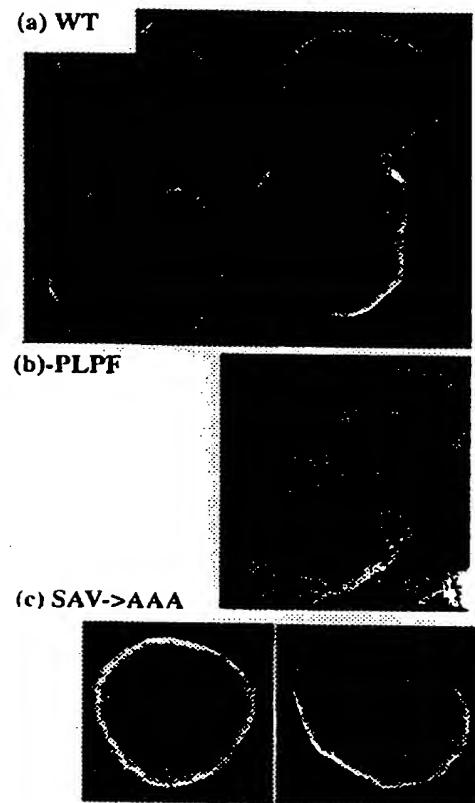


Figure 24

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